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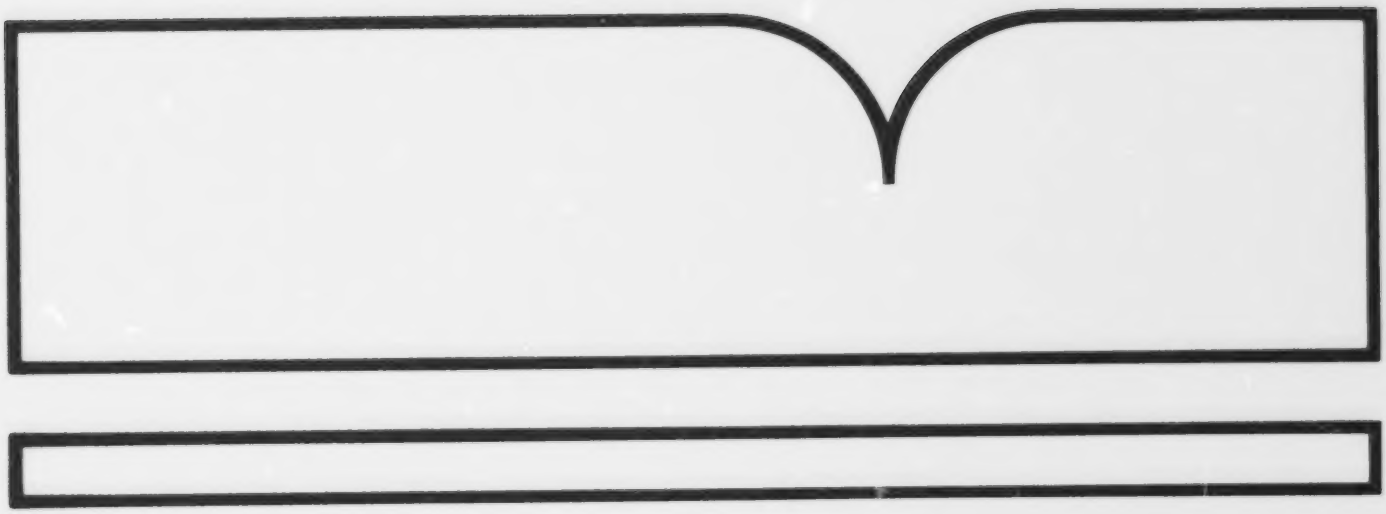

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16. ABSTRACT

Ashford's 1981 paper in BIOMETRICS provides a framework for interpreting the joint action of mixtures of drugs. These concepts are applied to the situation in which the mechanism of three drugs are fairly well understood and mixtures of pesticides and drugs are studied to aid in the understanding of the mechanism of certain types of pesticides.

Laboratory data is fit by nonlinear regression to models suggested by Ashford's paper. The conclusions drawn from fitting these models and the implication of the design of experiments for further studies are discussed.

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Using Ashford's General Model to Aid in the Understanding of a Pesticide's Neurotoxic Effect through Pesticide-Drug Mixture Experiments

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I. INTRODUCTION

Many changes can be measured in the brain after exposure to a neurotoxin. Some of these changes are not involved in the production of clinical symptoms of interest that these toxins cause. By learning the mechanism of action of a class of pesticides we are equipped with the necessary knowledge for determining the relevant *in vitro* tests for the assessment of risk to a particular disease state of interest.

Pyrethroid insecticides are potent neurotoxicants. They are classified as being either Type I or Type II based upon several factors: 1) *in vivo* toxic signs in rats (Verschoyle and Aldridge, 1980), mice (Lawrence and Casida,1982), and insects (Gammon et al, 1981); 2) duration of prolongation of sodium current in nerve axons (Narahashi, 1962; Lund and Narahashi, 1983); and 3) interactions at the γ-aminobutyric acid (GABA) receptor complex (Gammon et al., 1982); Lawrence and Casida, 1983). Structurally, the Type II pyrethroids usually contain an α-cyanophenoxybenzyl moiety, whereas the Type I pyrethroids lack this component. Currently, a major question is whether the observed *in vivo* effects of pyrethroids involve action primarily at the axonal sodium channel or the GABA receptor complex.

The aim of the research presented here was to determine the best statistical approach to use when analyzing behavioral data that was collected to assess the potential additivity of two compounds. Data was collected to determine whether an addivity of effects would result from the *in vivo* combination of low dosages of cismethrin (Type I) or deltamethrin (Type II), with compounds known to interact at the three major binding sites on the GABA receptor complex (e.g., picrotoxin, a GABA activated chloride antagonist; muscimol, a GABA receptor antagonist; and chlordiazepoxide, a benzodiazepine receptor agonist).

II. RESEARCH STRATEGY

The response variable was obtained after the rat is dosed by placing the rat in a figure-eight maze for one hour. The figure-eight maze consists of a series of interconnecting alleys (10 × 10 cm) converging on a central arena and covered with transparent acrylic plastic (Reiter et al, 1975). The maze is equipped with 8 photo-transistor/ photodiode pairs. Each interuption of the photobeam is one activity count. The counts per hour is the motor activity response variable.

Previous data has demonstrated decreased figure-eight maze activity following exposure to pyrethroids. These decreases in locomotor (ambulatory) activity are probably due to simultaneous increases in non-locomotor behaviors. Since the motoractivity response variable, as described above, is mainly a measure of locomotor activity, the response variable is likely to be negatively correlated with this form of nonlocomotor activity.

Figure 1 is a path diagram that summarizes some of these concepts in terms of the drugs and pesticides that we studied. Picrotoxin (PTX) is a drug that binds at a site on the GABA receptor complex. Through this binding it affects the chloride channel and causes convulsions. Recently, it has been found that Type II pyrethroid pesticides also bind at the same site on the GABA receptor complex as does PTX. Muscimol (MUS) and chlordiazepoxide (CPX) are two drugs that bind at other sites on the GABA receptor complex. The effect of these drugs is to open the chloride channel and they act as anticonvulsants. *In vitro* information is relatively complete at the channel stage of this diagram. Below the channel stage there is not much *in vitro* information and the path lines are much more speculative.

An isobologram is a plot of the contours of equal response (isobols) versus the dosage levels. Compounds that act as dilutions of one another (compounds that effect the same sites) will have straight line isobols when plotted versus the arithmetic dosage. Monotonic transformations of the dosage can change the shape of the isobols. If the dose-response curves of two compounds have different shapes as reflected by their logistic slope parameters being different, their isobols may not be straight lines. Different slopes may arise for a variety of reasons. One reason may be that the concentration of the compound reaching the site of action may not be proportional to the applied dose. A second reason might be that the compounds do not have the same sites of action. Also, the compounds might bind to the same sites, but have different effects at those sites.

The null hypothesis was that pairs of compounds would have straight line isobols, and a two step research strategy was employed to test that the ED50 isobol was a straight line. First, a series of experiments were carried out to estimate the ED50 of each compound. Secondly, mixture experiments were carried out to test for interactions between compounds. Two pairs of drugs with known sites of action were also studied to validate this approach. The first pair of drugs (haloperidol, HAL and chlorpromazine, CPZ) act at the same site and the second pair (chlorpromazine, CPZ and chlordiazepoxide, CPX) act at different sites. The design for the mixture experiments included the following 7 treatment groups: 1) a non-injected control; 2) an ED50 dosage of CPZ (2.0 mg/kg); 3) a dosage of CPZ equal to 75% of its ED50 (1.5 mg/kg), in combination with a dosage of HAL equal to 25% of its ED50 (0.031 mg/kg); 4) a dosage of CPZ equal to 50% of its ED50 (1.0 mg/kg), in combination with a dosage of HAL equal to 50% of its ED50 (0.062 mg/kg); 5) a dosage of CPZ equal to 25% of its ED50 (0.5

mg/kg), in combination with a dosage of HAL equal to 75% of its ED50 (0.094 mg /kg); 6) an ED50 dosage of HAL (0.125 mg/kg); 7) an ED50 dosage of both CPZ and HAL. The same design using CPZ and CPX were used to study their joint action.

A five point design was used to study the effects of mixtures of pesticides and GABAergic drugs. The five points were a control, ED50's of the pure compounds, and two mixture points on the straight line joining these estimated ED50's. One mixture point was 75% of the ED50 of the drug and 25% of the ED50 of the pesticide. The second mixture point was 75% of the ED50 of the pesticide and 25% of the ED50 of the drug. Later experiments used 67% and 33% of these ED50s in order to get uniform spacing between points. There were 6 mixture experiments involving one of the three drugs (CPX, MUS, or PTX) and one of the 2 pesticides (DLT or CSM). Ten rats were dosed at each different dosage and the rats were balanced between the 16 different mazes and with respect to other factors. The statistical analysis involved approximating the response curve between these four points by a cubic polynomial. The linear coefficient was regarded as a measure of the equivalence of the response at the two pure dosage levels, and the quadratic coefficient was regarded as an indicator of syngerism or antagonist (i.e., non-additivity). The cubic coefficient was regarded as a measure of lack of fit of the quadratic model. The pvalues for testing if these coefficients were zero are shown in Table 1.

A fourth degree polynomial was used to analyze the data from these two validation studies. Coefficients associated with terms higher than second degree were regarded as a lack of fit of the quadratic model. Plots of the means are shown in Figures 2 and 3. The p-values for testing if these coefficients are zero are listed in Table 2. None of the coefficients were significant for the fourth degree polynomial fit to the mixture data of the two drugs thought to act at the same site. Only the quadratic term was significant for fourth degree polynomial of the mixture data from the two drugs thought to act at different sites. Rather than considering a quadratic model, one could test the means for nonlinearity. This test did not agree with the polynomial analysis in that it was insignificant at the 5% level. In view of the obvious pattern of the means in Figure 3, it appears that the test for nonlinearity is insensitive.

III. ASHFORD MODELS

In an effort to better understand the mixture data, the general framework of Ashford's models (Ashford, 1981) was applied to these data. Figure 4 is an outline of Ashford's general framework. He considers components of stimuli and their levels. The elements of the z vector are the dosages in a particular mixture. The system S to which the stimuli are applied might be the central nervous system, although in his 1981 paper Ashford viewed this as a subsystem. Subsystems and sites of action are also considered. It is also considered that a site might be diffuse and belong to more than one subsystem. The function f_i expresses the effect that the stimuli have on the activity at the site, and is called the effective stimuli of the sites belonging to subsystem S_i. The function g_i is the sum of all the effective stimuli of the sites belonging to subsystem S_i, and is called the 'operative level' of subsystem S_i.

Ashford gave an example of the application of his general models to a situation that is very similar to the situation presented here. This application included the concept of occupancy theory and intrinsic activity. Occupancy theory assumes that if two drugs bind at the same site that they compete for receptor sites. Intrinsic activity reflects what effect binding has on the activity at the site, and is incorporated into the model by the parameter α. If α is less than one, the activity is decreased. If α is greater than one, the activity is increased, and if α is equal to one, there is no change in activity.

Figure 5 shows an application of the Ashford model to the mechanism of action if the sodium channel mechanism is used to explain the Type II effects. It should be noted that the effective stimuli of DLT, the Type II pyrethroid pesticide addresses the fact that DLT binds to the site, but since the intrinsic activity is assumed to be one, the chloride channel is not involved.

In Figure 6, A2 is the picrotoxin site on the GABA receptor complex. The chloride channel mechanism shown here assumes that the intrinsic activities of PTX and the Type II pyrethroid are equal in order to reduce the number of parameters to be estimated. Not shown are the slope parameters that were allowed to vary between compounds for both models. Also, the response variable was transformed to the log of the the activity plus one because the data seemed skewed when the mean activity was low.

These two models were fit to a combined data set. The combined data set was the data from the dose -reponse experiments and the mixture experiments involving PTX and the two pesticides. The two anticonvulsant drugs were excluded because one of the slopes was large, and because the reduced design had a symetry with respect to compounds known to act through the sodium channel (CSM) and those acting at the GABA receptor complex.

Both models fit the data about equally well. The largest difference in prediction was when PTX=2.5 mg/Kg and DLT=4.0 mg/Kg. This difference was about 12% of the control mean. Since this point represented an extrapolation point (possibly large variance of this estimated small, it did not seem worthwhile to do further experimentation to investigate this difference in order to identify the correct model. Box and Hill (1967) consider a Bayesian experimentation/model fitting strategy for discriminating between mechanistic models.

The Ashford models were also fit to the validation experimental data. For each pair of drugs the data consisted of the combined dose-response data

and the mixture data. The models fit and the results are shown in Table 3 for the case of the two drugs known to act at the same site. The six models fit were a one site and two site model with the slopes being either fixed at one, assumed to be equal, or allowed to vary between drugs. There was no significant lack of fit for any of these models. Since the null hypothesis is that the compounds act as dilutions, this suggests that the one site model is adequate to explain the data. Thus, the strategy is validated to a certain extent. However, it appears that the discrimination power of the strategy is weak.

In Table 4 are the results of fitting the Ashford models to the two drugs acting at different sites. The same models were used as in the case of the two drugs acting at the same site. This data was not all obtained on the same day, and the Wilcoxon test suggested that the median activity of data obtained on different days was significantly different from those observations obtained different days, but at the same dosage. Attempting to simultaneously model an apparent dosing error and the Ashford model resulted in a very ill conditioned situation. Therefore, the mixture dosages were adjusted so that the response means of dose-response data agreed with the mixture data before the Ashford models were fit to the data. Of the six models, the only models not rejected by the data were the two site models with a common slope and with the slopes not equal. To achieve adequate fits in fitting the Ashford model to these type of data, these results suggest that it is at least necessary to consider that the slopes may not equal one.

IV. EXPERIMENTAL DESIGN CONSIDERATIONS

Two designs are considered for future studies of this type. We consider the situation where one is interested in determining whether the mechanism of action of pesticide A is similar to drug B or drug C. The two designs considered are a simplex-type design in an attempt to bracket the ED50 isobol and an orthogonal design. Since it might not be possible to complete the experiment simultaneously, it is necessary to have an idea of what the between experiment variability is. This was estimated from the activity of the control rats used in the experiments discussed here. A mixed model was fit to this control data. The fixed effect was age which tended to vary between experiments. This analysis indicated that between experiment variability was 25% of within experiment variability.

The simplex-type design considered is shown in Figure 7. The designs for 2/3 times the ED50 plane and the design for the ED50 plane are called Simplex Lattices (Scheffe', 1958). The orthogonal design is a 27 point design. This design includes all combinations of zero, half and one times the ED50. Scheffe' type polynomials (Scheffe', 1958) were considered as the true response surface.

The design criterion adopted was to obtain half-widths on the 95% geometric ratio confidence surface of the ED50 isobol to be less than 10%. This criterion was met by the simplex-type design if 3 rats were used at each point and the experiments were replicated 10 times. The criterion was also met by the orthogonal design using the above numbers of rats and replicates.

V. CONCLUSIONS

Our preference is for the simplex type of designs. The results of fitting polynomials to these types of designs are understandable to many nonmathematically-oriented researchers. However, these designs can't be employed in all situations. For example, if one drug has no effect when administered alone, the ED50 isobol would not intersect the axis of that drug. In this case, orthogonal designs would probably be needed.

The data obtained in this study was quite minimal and the study is best viewed as a screening study for mechanisms. Therefore, the results of fitting the Ashford models may be misleading if compared to a study where the design was more appropriate for the fitting of such models. For example, the dose-response data was obtained for the purpose of estimating the ED50, and only a few dosages were studied. More complete doseresponse data might have provided better estimates of the slopes and the intrinsic activity. Simultaneous estimation of all parameters is a preferred approach. However, simultaneous estimation resulted in convergence problems when both between replicate parameters and the parameters of the Ashford model were being iterated upon. A two stage approach could involve adjusting out nuisance parameters before going on to the second stage of parameter estimation for the Ashford models. It might be that an Emperical Bayes (Casella, 1985) approach would be useful in making these adjustments. For example, rather than dividing by the control means of different replicates, one could divide by the Emperical Bayes estimates of these means.

However, simultaneous estimation should have been used in a couple of instances. One instance was the modeling of the dose-response curves. Such modeling might be useful in selecting only for subsequent mixture studies, those drugs or pesticides with similar shape dose-response curves. The second instance was the fitting of the validation data to the Ashford models. In that case, one of both pairs of drugs was the same drug, CPZ. Simultaneous estimation would have permitted testing if that drug's mechanism of action was similar to the mechanism of action of one of the other pairs of drugs. This less global but very relevant hypothesis may have better shown that the Ashford model is sensitive enough to discriminant between modes of action of these drugs.

VII. REFERENCES

Ashford,J.R.(1981). "General models for the joint action of drugs", Biometrics,37,457-474.

Box,G.E.P and Hill,W.J.(1967). "Discrimination among Mechanistic Models", Technometics,9,57-71.

Claringbold,P.J.(1955). "Use of the simplex design in the study of the joint action of related hormones", Biometrics,11,174-185.

De Lean,A.,Munson,P.J.,and Rodbard,D.(1978). "Simultaneous analysis of Families of Sigmoidal Curves: application to bioassay, and physiological dose-response curves", Am. J. Physiol. 235(2):E97-E102.

Draper,N.R. and Smith,H., Applied Regression Analysis, John Wiley and Sons,New York 1966.

Fedeli,L., Meneghini,L., Sangiovanni,M., Scrollini ,F., and Gori,E.(1972). "Quantitative evaluation of joint drug action", Toxicological Problems of Drug Combinations, Proceedings of the European Society for the Study of Drug Toxicity, Vol. XIII, Excerpta Medica Foundation, Amsterdam.

Gammon,D.W.,Brown,M.A., and Casida,J.E.(1981). "Two classes of pryethroid action in the cockroach",Pestic. Biochem. Physiol.,15,181-191.

Gammon,D.W., Lawrence,L.J., and Casida,J.E. (1982). "Pyrethroid toxicology: Protective effects and phenobarital in the mouse and cockroach", Toxicol. Appl. Pharmacol.,66,290-296.

Gessner,P.K.(1974). "The isobolographic method applied to drug interactions",In: Morselli,P.L. et al (eds) Drug Interactions, Raven Press, NY.

Goldstein,A., Aronow,L., and Kalman,S.M., Principles of Drug Action - The Basis of Pharmacology, Harper and Row, New York 1969.

Lawrence,L.J. and Casida,J.E.(1982). "Pyrethroid Toxicology: Mouse intracerebral structure-activity relationships", Pestic. Biochem. Physiol. 18:9-14.

Lawrence,L.J. and Casida,J.E.(1983) "Stereospecific action of pyrethroid insecticides on the γ-aminobutyric acid receptor-ionophore complex", Science 221:1399-1401.

Loewe,S.(1953). "The problem of synergism and antagonism of combined drugs" Arnz-Forsch 3:285-290.

Lund,A.E. and Narahashi,T.(1983). "Kinetics of sodium channel modication as a basis for the variation in the nerve membrane effects of pyrethroids and DDT analogs", Pestic. Biochem. Physiol. 20,203-216.

Narahashi,T.(1962). "Effect of the insecticide allethrin on membrane potential of cockroach giant axons", J. of Cell. Comp. Physiol.,59,61-65.

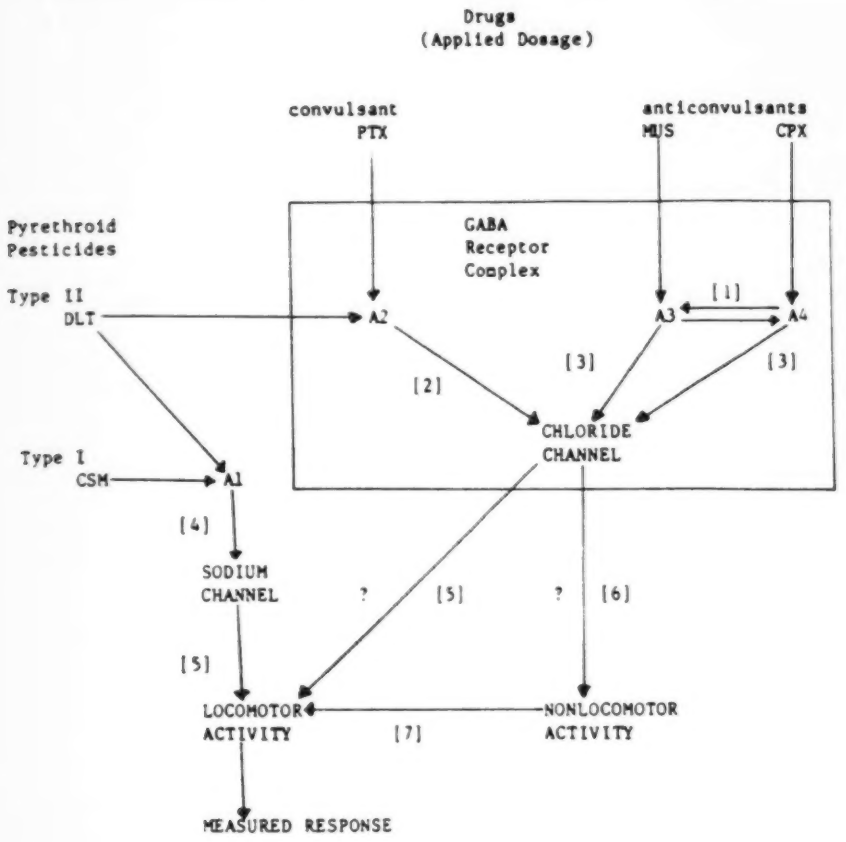
Reiter,L.W., Anderson,G.E., Laskey,J.W., and Cahill, D.F.(1975). "Development and Behavior changes in the rat during chronic exposure to lead", Environ. Hlth. Perspect.,12, 119-123.

Scheffe',H.(1958). "Experiments with Mixtures", J. Roy. Statist. Soc.,B,20,344-360.

Verschoyle,R.D. and Aldridge,W.N.(1980). "Structure-activity relationships of some pyrethroids in rats",Arch. Toxicol., 45,325-329.

FIGURE 1

A Concept of the Mechanism of Action of Pyrethroid Pesticides



Legend of paths

[1] Presence of one drug increases the affinity at the other site
[2] Channel blocker
[3] Opens channel
[4] Formation of modified open state of sodium channel
[5] Effect decreases locomotor activity
[6] Blocked channel increases nonlocomotor activity
[7] Negative correlation

Figure 2

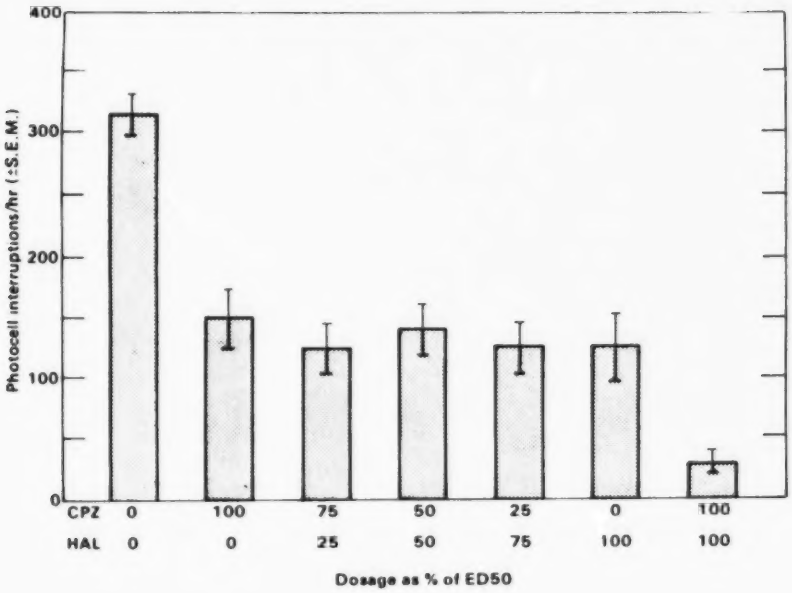


Figure 3

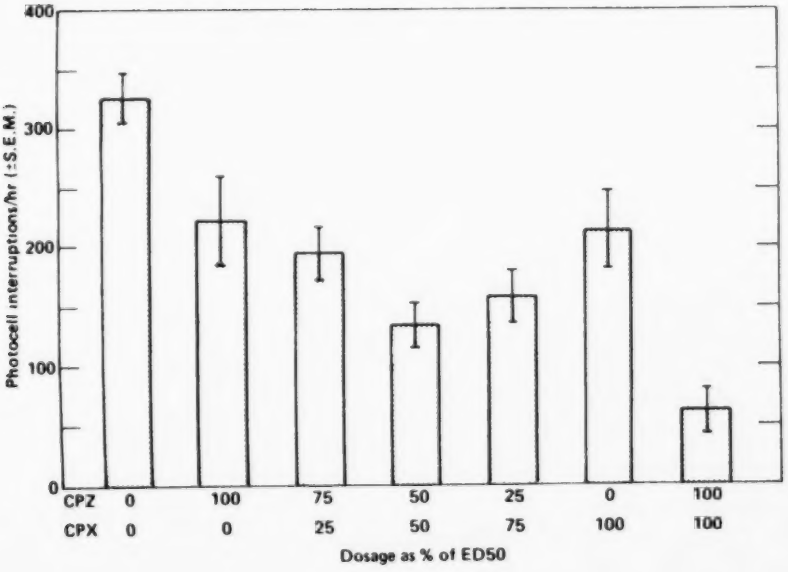
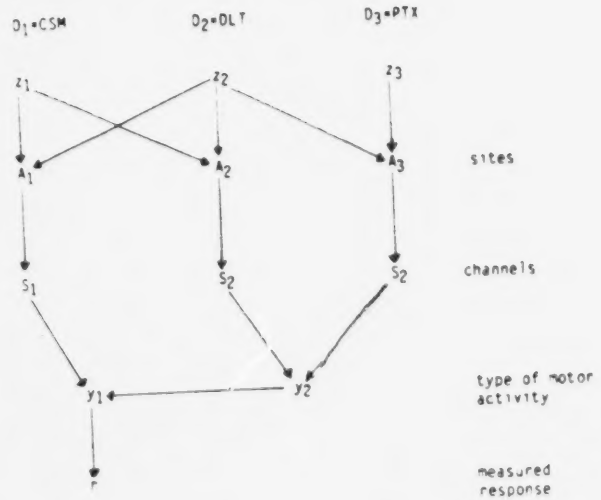


FIGURE 4
An Outline of Ashford's General Framework

Symbols		Interpretation
$D_1, D_2, \ldots, D_d$		Components of Stimulus
$z_1, z_2, \ldots, z_d = \underline{z}$		Levels of components of stimulus (applied dose)
S		System to which stimuli are applied
Relationship determined by the way that a system is constructed and functions	$S_1, S_2, \ldots, S_k$	Subsystems of S
	$A_1, A_2, \ldots, A_m$	Sites of action - may be localized or diffuse (belong to more than one subsystem)
$f_l(\underline{z})$ $l=1,2,\ldots,m$		Effective stimulus - Involves transfer of drug to the site of action and the mechanism of the drug at the site of action itself. $f_l(\underline{z})$ is monotone.
$g_i(\underline{z}) = \sum_{\substack{\text{all} \\ A_k \in S_i}} f_k(\underline{z})$		'Operative level' of the stimulus in relationship to subsystem S_i. Assumes g_i is the sum of the effective stimulus at each site.
$\underline{y} = (y_1, y_2, \ldots, y_k)$		Composite response of the whole system consists of underlying response of each subsystem.
$\underline{r} = \underline{m}(\underline{y})$		Observable response
$y_i = x_i(\underline{z}) + \varepsilon_i$		'Effects function' x_i is the same for all rats in a strain. ε_i is the individual variability (susceptibility or tolerance)
$x_i = h_i(g_i(\underline{z}))$		Each component of $\underline{x}$ is a monotone increasing function $h_i(g_i)$ of the operative level of the stimuli.

FIGURE 5
Ashford Model of the Sodium Channel Concept



$f_1(z_1,z_2) = \log(1/1+a_{11}z_1+a_{12}z_2)$

$f_2(z_1,z_2) = \log(1/1+a_{21}z_1+ a\ a_{22}z_2)/(1+a_{21}z_1+a_{22}z_2))$

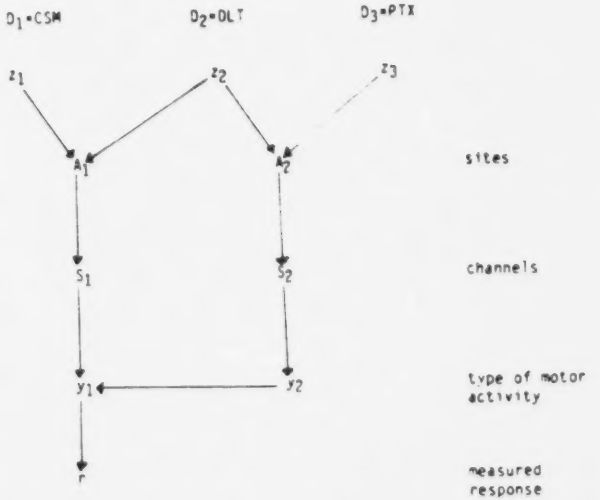
$f_3(z_2,z_3) = \log(1/1+a_{32}z_1+ a\ a_{22}z_2)/(1+a_{32}z_2+a_{33}z_3))$

$g_1(\underline{z}) = f_1 \qquad g_2(\underline{z}) = f_2 \cdot f_3$

$y_1 = h_1(g_1) \qquad y_2 = h_2(g_2)$

$r = y_1 \cdot r_1 y_2$

FIGURE 6
Ashford Model of the Chloride Channel Concept



$f_1(z_1,z_2) = \log(1/1+a_{11}z_1+a_{12}z_2)$

$f_2(z_1,z_2) = \log(1/1+ a\ a_{21}z_1+ a\ a_{22}z_2)/(1+a_{21}z_1+a_{22}z_2))$

$g_1(\underline{z}) = f_1 \qquad g_2(\underline{z}) = f_2 \cdot f_3$

$y_1 = h_1(g_1) \qquad y_2 = h_2(g_2)$

$r = y_1 \cdot r_1 y_2$

FIGURE 7
Simplex type designs to Bracket the ED50

	Surface	Points (proportion of ED50)	Number of Points
.	Control	(0,0,0)	1
. .	(2/3) ED50	(.67,0,0) (0,.67,0) (0,0,.67) (.33,.33,0) (.33,0,.33) (0,.33,.33)	6
. . .	ED50	(1,0,0) (0,1,0) (0,0,1) (.67,.33,0) (.33,.67,0) (.67,0,.33) (.33,0,.67) (0,.67,.33) (0,.33,.67) (.33,.33,.33)	10
. . . .	(4/3) ED50	(1.33,0,0) (0,1.33,0) (0,0,1.33) (1,.33,0) (.67,.67,0) (.33,1,0) (1,0,.33) (.67,0,.67) (.33,0,1) (0,1,.33) (0,.67,.67) (0,.33,1) (.67,.33,.33) (.33,.67,.33) (.33,.33,.67)	15
		total	32

Model:

Quadratic polynomial model of Sheffe' type

TABLE 1
P-values from fitting a cubic polynomial to the drug-pesticide mixture means

Drugs	Test of Coefficient	Pesticides Type I CSM	Pesticides Type II DLT
PTX	linear	0.0677	0.4993
	quadratic	0.0594	0.1685
	cubic	0.9633	0.5885
MUS	linear	0.6639	0.0098
	quadratic	0.0020	0.0001
	cubic	0.0590	0.0017
CPX	linear	0.0012	0.0004
	quadratic	0.0065	0.0974
	cubic	0.4362	0.0300

TABLE 2
P-values of fitting a fourth degree polynomial to validation study data

Test of Coefficient	Drugs known to act at same site	Drugs known to act at different sites
linear	0.4457	0.5282
quadratic	0.7766	0.0233
cubic	0.7381	0.4091
quartic	0.5945	0.4542

TABLE 3
Variance of Residual Error from Fitting
Various Ashford models to data of two drugs
thought to act at same site

Constraint on Slopes	Number of Sites One	Number of Sites Two	Number of parameters in the model
Slopes are equal to one	0.4131	0.4024	3
Slopes are equal	0.3990	0.3762	4
Slopes are not equal	0.4013	0.3778	5

Variance of Pure Error = 0.3523

Approximate F test for Lack of Fit of the One slope model with the slopes constrained to equal one: (Smith and Draper,1966)

$$F_{3,144} = \frac{(162-3)\times 0.4131-(162-18)\times 0.3523}{15\times 0.3523}$$

$$= 0.68$$

Models:

One Site

$$\log_e(\text{activity}+1) = \log_e C_i/(1+a_1w_1+a_2w_2)$$

Two Site

$$\log_e(\text{activity}+1) = \log_e C_i/(1+a_1w_1)(1+a_2w_2)$$

where $w_j=\exp(s_j \log z_j)$ $j=1$ or 2

TABLE 4
Variance of residual error from fitting various
Ashford models to data of Two Drugs thought
to act at different sites.

Constraint on Slopes	Number of Sites One	Number of Sites Two	Number of parameters in the model
Slopes are equal to one	* 0.2614 $F_{15,149}$=2.56	0.2523 $F_{15,149}$=2.13	3
Slopes are equal	0.2489 $F_{14,149}$=2.03	0.2322 $F_{14,149}$=1.18	4
Slopes are not equal	0.2504 $F_{13,149}$=1.93	0.2336 $F_{13,149}$=1.03	5

* F values for testing the lack of fit. Variance of pure error was 0.2287. The 95 percentile of an F with 15 and 120 degrees of freedom is 1.67.